PB82-207820

Ammonia Toxicity to Fishes. Effect of pH on the Toxicity of the Un-ionized Ammonia Species

Montana State Univ.
Bozeman

Prepared for

Environmental Research Lab.-Duluth, MN

Jul 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completir

1. REPORT NO.	2.	3. PB82-207820
EPA-600/J-81-550	Journal Article	

4. TITLE AND SUBTITLE	5. REPORT DATE
Ammonia Toxicity to Fishes. Effect of pH on the Toxicity of the Un-ionized Ammonia Species	May 1982
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Thurston, R. V., R. C. Russo, and G. A. Vinogradov	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Fisheries Bioassay Laboratory Montana State University Bozeman, Montana 59717	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, Minnesota 55804	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES
Environ. Sci. Technol. 15: 837-840 (1981)

16. ABSTRACT

United States and European water quality criteria to protect aquatic life assume that the toxicity of aqueous ammonia is attributable to the un-ionized (NH_3) chemical species, and that the ionized (NH_4^+) species is nontoxic or significantly less toxic than NH_3. Data are presented here on the toxicity of ammonia to rainbow trout (Salmo gairdneri) and to fathead minnows (Pimephales promelas) from two series of 96-h flow-through toxicity tests in which pH was controlled within the range 6.5-9.0. Results show that the toxicity of ammonia in terms of NH_3 increased at lower pH values. It is concluded that NH_4^+ exerts some measure of toxicity and/or that increased H^+ concentration increases the toxicity of NH_3. It is recommended that water quality criteria be revised to consider the pH dependence of the toxicity of ammonia.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	4
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-81-550
Journal Article

Reprinted from ENVIRONMENTAL SCIENCE & TECHNOLOGY, Vol. *15*, Page 837, July 1981
1981 by the American Chemical Society and reprinted by permission of the copyright owner

Ammonia Toxicity to Fishes. Effect of pH on the Toxicity of the Un-ionized Ammonia Species

Robert V. Thurston* and Rosemarie C. Russo

Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana 59717

German A. Vinogradov

Institute for Biology of Inland Waters, Academy of Sciences, Borok, Jaroslavl, Oblast, U.S.S.R.

■ United States and European water quality criteria to protect aquatic life assume that the toxicity of aqueous ammonia is attributable to the un-ionized (NH_3) chemical species, and that the ionized (NH_4^+) species is nontoxic or significantly less toxic than NH_3. Data are presented here on the toxicity of ammonia to rainbow trout (*Salmo gairdneri*) and to fathead minnows (*Pimephales promelas*) from two series of 96-h flow-through toxicity tests in which pH was controlled within the range 6.5–9.0. Results show that the toxicity of ammonia in terms of NH_3 increased at lower pH values. It is concluded that NH_4^+ exerts some measure of toxicity and/or that increased H^+ concentration increases the toxicity of NH_3. It is recommended that water quality criteria be revised to consider the pH dependence of the toxicity of ammonia.

Introduction

Ammonia is a major toxicant to fishes and other aquatic life. It enters natural water systems from several sources including industrial wastes, sewage effluents, coal gasification and liquefaction conversion process plants, and agricultural discharges including feedlot runoff. It is also a metabolic waste product of fishes, and as such can present a major problem in fish culture.

In aqueous solutions, ammonia assumes two chemical species as shown by the following equation.

$$NH_3(g) + nH_2O(l) \rightleftarrows NH_3 \cdot nH_2O(aq) \rightleftarrows NH_4^+ + OH^- + (n-1)H_2O(l)$$

These species are the gaseous or un-ionized form (NH_3), bound to at least three water molecules (*1*), and the ionized form (NH_4^+). Early reported research on the toxic effects of ammonia to fishes (*2–4*) implicated NH_3 as being toxic, and NH_4^+ was considered nontoxic or appreciably less toxic. The explanation for the observations leading to this conclusion is the ease of NH_3 diffusion across fish gill membranes which are much less permeable to NH_4^+. NH_4^+, however, is excreted across the gills via a carrier-mediated process in exchange for Na^+. The aqueous ammonia equilibrium is strongly dependent upon the pH of the solution, and to a lesser extent upon temperature and ionic strength. As pH increases, increasing the hydroxide ion concentration, the equilibrium shift is toward the NH_3 species. Within the pH range acceptable to most freshwater fishes, an increase of one pH unit will increase the NH_3 concentration ~10-fold. Temperature increase also favors the NH_3 species, but to a lesser extent; ionic-strength increase, at low concentrations, favors the NH_4^+ species (*5*, *6*). On the basis of the assumption that NH_3 alone is toxic, and because of the approximately 10-fold increase in the percentage of NH_3 with an increase of one pH unit, it follows that

the same amount of total ammonia would be ~10 times more toxic to fishes at pH 8.5 than at 7.5. Such has proven to be the case in several independent studies. Because of these observations, European and United States water quality criteria for ammonia are stated in terms of un-ionized ammonia (*7, 8*); these stated criteria are recommended for the entire pH range (6.5–9.0) considered safe for freshwater aquatic life (*8*).

Earlier assumptions to the contrary, recent studies (*9–13*) indicate that pH plays a more significant role in the toxicity of ammonia than simply that of controlling the NH_3/NH_4^+ equilibrium in water. The present study was undertaken to measure the acute toxicity of ammonia to two species of fishes over the entire range recommended by the present U.S. EPA criterion for pH.

Methods

Nine 96-h flow-through toxicity tests were conducted on rainbow trout and six on fathead minnows. The rainbow trout were Beulah (Wyoming) strain, obtained from the Fish Cultural Development Center (FCDC), U.S. Fish and Wildlife Service (FWS), Bozeman, MT; they were between 4 and 5 months old at the time of testing. The fathead minnows were adults obtained from a pond near Billings, MT, which had been stocked 5 yr earlier from a farm pond in eastern Montana. The fathead minnows were maintained for 2 months prior to testing, and the rainbow trout from day of hatching, in water obtained from a ground spring at FCDC; the dilution water for the toxicity tests was from this same source. Chemical characteristics of this water are listed in Table I.

Before testing, the rainbow trout were fed a FWS hatchery production diet (Rangen, Inc., Buhl, ID) and the fathead minnows were fed this same diet supplemented occasionally with live brine shrimp, 3 times daily; fish were not fed while under test. Fish (10 per tank) were weighed collectively at the beginning of the tests, and total lengths were measured individually as mortalities occurred or at test termination. Mean size (and range) for the test groups of rainbow trout were 9.5 (8.0–11.1) g and 9.4 (9.2–9.9) cm; for fathead minnows these were 1.9 (1.8–2.0) g and 5.2 (4.7–5.6) cm.

Toxicant solution was delivered to the test tanks (water volume, 60 L each) by proportional diluters (*14*) at a flow rate of 500 mL at 2–3-min intervals to each of six tanks (five test and one control). Water replacement time in the tanks was ~5 h. Reagent-grade ammonium chloride was used as the toxicant, and dilute solutions of reagent-grade sodium hydroxide or hydrochloric acid were used to maintain the desired pH in the control and test tanks. The acid or base solutions were metered into the proportional diluter discharge lines by means of micrometering pumps and were flushed into the tanks each time the diluters discharged. The pH of each tank was monitored 2–4 times daily, and the flow rates from the acid/base metering pumps were adjusted as needed.

For each fish species, tests were conducted on successive weeks, three at a time: one acid, one base, and one at the normal pH (7.8–7.9) of the test dilution water. The test at normal pH was repeated each week to verify that test conditions from week to week were comparable and that the stock of test fish had not changed appreciably during the period of time over which the tests were conducted. Before the start of each test, fish were placed in the test tanks in water of normal pH. Adjustments in pH to the desired test pH conditions were made gradually over a 24-h period, and the fish were then maintained at the test pH for 48 h prior to starting the test. Toxicant solution was added to the test tanks starting at the beginning of the 96-h test period; 50% test concentration was effectively reached in each tank within 5 h from the start of the test, and full test concentration within 20 h.

Chemistry analyses were performed on water in the tanks by using standard methods (*15, 16*). During each test, simultaneous measurements of total ammonia nitrogen, pH, and temperature were made in each tank between 5 and 8 times after full test concentration was reached. Averaging of pH was accomplished with a computer program which converts pH values to their corresponding hydrogen ion concentrations, averages these, and then reconverts the average to pH units. Un-ionized ammonia concentrations were calculated for each tank (*5, 6*) from measured total ammonia nitrogen, pH, and temperature. Characteristics of the test water (overall mean and range of means for the separate toxicity tests) for the following variables were measured in mg/L during the rainbow trout tests: dissolved oxygen, 7.8 (7.1–8.6); nitrate nitrogen, 0.07 (0.01–0.11); nitrite nitrogen, 0.00; and hardness as $CaCO_3$, 200 (181–206). These characteristics for the fathead minnow tests were as follows: dissolved oxygen, 9.6 (8.9–10.6); nitrate nitrogen, 0.07 (0.04–0.13); nitrite nitrogen, 0.00; and hardness as $CaCO_3$, 182 (178–189). Mortality observations were made at 4–8-h intervals during the first 24 h of each test and at 6–12-h intervals during the next 72 h. There was one control mortality at 88 h during test 555 (rainbow trout); there were no control mortalities in the other tests. The 96-h median lethal concentrations (LC50) and their 95% confidence intervals were calculated for each test from the average (mean) total ammonia-nitrogen and un-ionized ammonia-nitrogen values in each tank by using the Trimmed Spearman-Karber method (*17*).

Table I. Chemical Characteristics of the Dilution Water Used in Bioassays [a]

Al	<1	Ni	<0.005
As	0.0012	Pb	<0.015
Cd	<0.005	Se	0.00085
Cr	<0.005	Zn	0.01
Cu	0.007	F^-	0.35
Fe	0.004	PO_4^{3-}	0.05
Hg	<0.00005	SO_4^{2-}	17.2
K	0.82	total organic carbon	3.3
Mg	16.7	SEC, μS (25 °C)	340
Mn	0.002	turbidity, NTU	1.6
Na	2.5		

[a] All values are in mg/L unless otherwise noted.

Results and Discussion

The 96-h LC50 values and their 95% confidence intervals for the acute toxicity tests are reported in terms of both total and un-ionized ammonia nitrogen (Table II). Average (mean) temperature and pH values in all tanks for each test are also reported because of the importance of these variables in the aqueous ammonia equilibrium; alkalinity is reported because it was a major variable dependent upon pH. The ranges reported for these variables are the extreme high and low measured values from all tanks for each test.

In both series of tests there was excellent reproducibility of LC50 results in the tests which were repeated at the normal pH (7.8–7.9) of the test water. Plots of the 96-h LC50 values (and their 95% confidence intervals) in terms of both total ammonia nitrogen and un-ionized ammonia nitrogen vs. pH for the rainbow trout tests are shown in Figure 1; these same plots for the fathead minnow tests are shown in Figure 2. From the data presented, fathead minnows would appear to be 1.5–3 times more tolerant than rainbow trout to the acutely toxic effects of ammonia.

Table II. Acute Toxicity of Ammonia to Rainbow Trout and Fathead Minnows at Different pH Values [a]

test no.	alkalinity, mg/L $CaCO_3$	temp., °C	pH	96-h LC50 (95% CI [b]) total NH_3 N, mg/L	96-h LC50 (95% CI [b]) un-ionized NH_3 N, mg/L
			Rainbow Trout		
557	75.1 (62.0–86.0)	14.1 (13.4–14.9)	6.51 (6.37–6.71)	161 (138–188)	0.130 (0.116–0.146)
552	107 (99.0–118)	14.1 (13.4–14.8)	6.80 (6.65–6.95)	100 (88.2–114)	0.152 (0.134–0.172)
555	149 (147–149)	14.0 (13.2–14.8)	7.30 (7.23–7.46)	72.7 (65.7–80.4)	0.374 (0.349–0.401)
551	177 (176–177)	13.2 (12.7–13.6)	7.82 (7.75–7.88)	32.9 (31.2–34.8)	0.523 (0.491–0.557)
554	176 (175–179)	12.9 (12.2–13.7)	7.84 (7.80–7.91)	33.0 (29.6–36.7)	0.513 (0.461–0.572)
559	175 (174–176)	13.0 (12.5–13.5)	7.87 (7.76–7.92)	34.4 (30.7–38.6)	0.588 (0.522–0.663)
556	188 (188–188)	14.1 (13.7–14.4)	8.29 (8.24–8.40)	13.0 (11.9–14.2)	0.658 (0.592–0.730)
553	193 (192–195)	13.9 (13.5–14.3)	8.82 (8.72–8.89)	3.98 (3.45–4.59)	0.563 (0.476–0.665)
558	196 (194–198)	14.5 (14.0–14.9)	9.01 (8.91–9.11)	2.53 (2.30–2.78)	0.534 (0.485–0.587)
			Fathead Minnows		
560	90.0 (86.2–92.0)	13.0 (12.4–14.0)	6.51 (6.42–6.62)	254 (222–290)	0.198 (0.175–0.224)
563	133 (132–134)	13.8 (12.8–15.0)	7.01 (6.90–7.08)	148 (138–159)	0.372 (0.346–0.400)
565	163 (162–164)	12.0 (11.2–12.8)	7.82 (7.74–7.93)	67.0 (59.3–75.7)	0.888 (0.800–0.986)
562	168 (168–169)	11.8 (11.1–12.5)	7.83 (7.76–7.92)	47.1 (42.0–52.7)	0.653 (0.594–0.718)
564	175 (174–177)	13.5 (12.7–14.0)	8.51 (8.40–8.66)	18.4 (16.3–20.8)	1.38 (1.23–1.56)
561	185 (184–187)	13.2 (12.7–13.7)	9.03 (8.91–9.17)	5.93 (5.07–6.92)	1.21 (1.03–1.41)

[a] Values reported for alkalinity, temperature, and pH are mean values of all measurements in all tanks with ranges in parentheses. [b] CI = confidence interval.

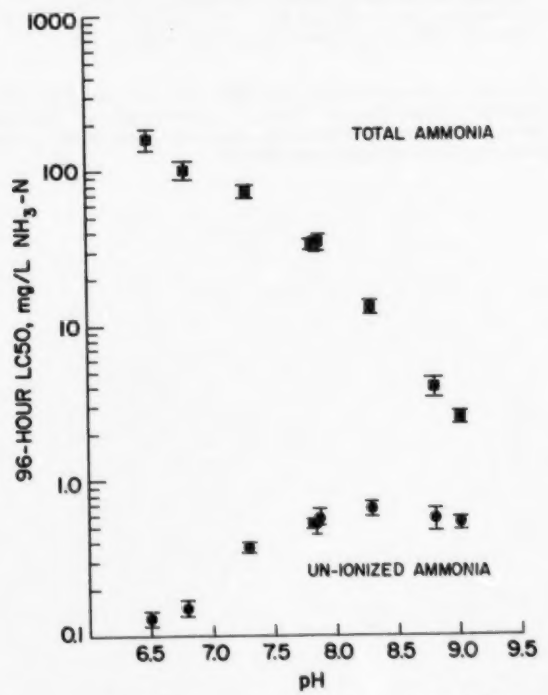

Figure 1. Acute toxicity of ammonia to rainbow trout: 96-h LC50 vs. pH (error bars are 95% confidence intervals).

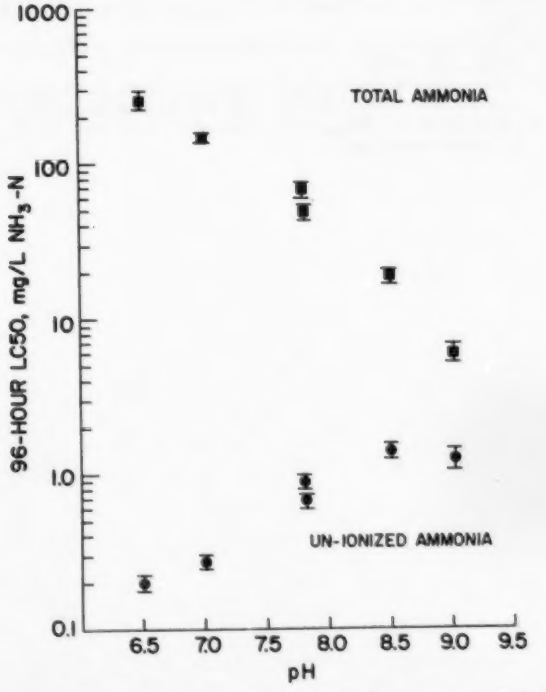

Figure 2. Acute toxicity of ammonia to fathead minnows: 96-h LC50 vs. pH (error bars are 95% confidence intervals).

If the un-ionized form of ammonia were solely responsible for the toxic action on the test fish, then one would expect that the un-ionized ammonia LC50 values would be reasonably constant for all tests on a single fish species, regardless of the solution pH and total ammonia present. Examination of the data and the plots for both fish species shows that this is not the case. The maximum LC50 values in terms of un-ionized ammonia nitrogen occur within the pH range 8.0–8.5; the values at pH 6.5 are only 30% of these or less. A lower LC50 value indicates greater toxicity of the toxicant being tested.

It is not readily apparent from the data available whether the un-ionized ammonia-nitrogen LC50 values above pH 8 reach a peak and then decrease, or whether they plateau. It is apparent, however, that the un-ionized ammonia-nitrogen LC50 values for both series of tests are markedly less at low pH than they are at high. The simplest possible explanation for this would appear to be that the toxicity of NH_3 is not constant over the pH range tested, i.e., that increased H^+ concentration increases the toxicity of NH_3. A second explanation is that NH_4^+ exerts a toxic effect; at concentrations

below ~20–50 mg/L total ammonia nitrogen, the effect of NH_4^+ is overridden by the effect of the much more toxic NH_3 fraction, but at higher concentrations of total ammonia the toxic effect of NH_4^+ can be noticed. If this is the case, the data would indicate that NH_3 is 300–400 times more toxic than NH_4^+ on a molecular comparison basis. Other factors, not noted here, may be operative.

It has been demonstrated by this and the studies cited earlier that the toxicity to fishes of ammonia in terms of un-ionized ammonia alone does not remain constant over the pH range considered acceptable to freshwater aquatic life. It would therefore seem advisable that water quality criteria to protect aquatic life be based either on un-ionized ammonia in combination with pH or on total ammonia in addition to un-ionized ammonia.

Acknowledgment

We thank Charles Chakoumakos and Kenneth Wang for contributions to the test apparatus design and for performing many of the chemical analyses. Victor T. Komov, Victoria E. Matai, and Boris A. Flerov provided helpful comments during the course of this research, and David J. Randall, William A. Spoor, and Kenneth E. F. Hokanson during preparation of the manuscript. Tanya Nezabilovskey provided a necessary link in communications at Sunoga Laboratory in Borok during discussions about the research.

Literature Cited

(1) Butler, J. N. "Ionic Equilibrium"; Addison-Wesley, Reading, MA, 1964.
(2) Chipman, W. A., Jr. Ph.D. Thesis, University of Missouri, Columbia, MO, 1934.
(3) Wuhrmann, K.; Zehender, F.; Woker, H. *Vierteljahrsschr. Naturforsch. Ges. Zürich* **1947,** *92*, 198–204 (in English translation).
(4) Wuhrmann, K.; Woker, H. *Schweiz. Z. Hydrol.* **1948,** *11*, 210–44 (in English translation).
(5) Emerson, K.; Russo, R. C.; Lund, R. E.; Thurston, R. V. *J. Fish. Res. Board Can.* **1975,** *32*, 2379–83.
(6) Thurston, R. V.; Russo, R. C.; Emerson, K. "Aqueous Ammonia Equilibrium—Tabulation of Percent Un-ionized Ammonia", EPA-600/3-79-091; Office of Research and Development, U.S. Environmental Protection Agency: Duluth, MN, 1979.
(7) European Inland Fisheries Advisory Commission. "Water Quality Criteria for European Freshwater Fish. Report on Ammonia and Inland Fisheries"; EIFAC Technical Paper No. 11, 1970. (Also in *Water Res.* **1973,** *7*, 1011–22.)
(8) U.S. Environmental Protection Agency. "Quality Criteria for Water"; Office of Water and Hazardous Materials, U.S. Environmental Protection Agency: Washington, DC, 1977.
(9) Armstrong, D. A.; Chippendale, D.; Knight, A. W.; Colt, J. E. *Biol. Bull. (Woods Hole, Mass.)* **1978,** *154*, 15–31.
(10) Robinson-Wilson, E. F.; Seim, W. K. *Water Resour. Bull.* **1975,** *11*, 975–86.
(11) Tabata, K. *Bull. Tokai Reg. Fish. Res. Lab.* **1962,** *34*, 67–74 (in English translation).
(12) Thurston, R. V. in "Proceedings of the Third USA–USSR Symposium on the Effects of Pollutants Upon Aquatic Ecosystems. Theoretical Aspects of Aquatic Toxicology", EPA-600/9-80-034; Office of Research and Development, U.S. Environmental Protection Agency: Duluth, MN, 1980; pp 118–37.
(13) Tomasso, J. R.; Goudie, C. A.; Simco, B. A.; Davis, K. B. *Trans. Am. Fish. Soc.* **1980,** *109*, 229–34.
(14) Mount, D. I.; Brungs, W. A. *Water Res.* **1967,** *1*, 21–9.
(15) American Public Health Association, American Water Works Association, Water Pollution Control Federation. "Standard Methods for the Examination of Water and Wastewater", 14th ed.; American Public Health Association: Washington, DC, 1976.
(16) U.S. Environmental Protection Agency. "Methods for Chemical Analysis of Water and Wastes", EPA-625/6-74-003; Methods Development and Quality Assurance Research Laboratory, U.S. Environmental Protection Agency: Cincinnati, OH, 1974.
(17) Hamilton, M. A.; Russo, R. C.; Thurston, R. V. *Environ. Sci. Technol.* **1977,** *11*, 14–9; correction, *ibid.* **1978,** *12*, 417.

Received for review February 24, 1981. Accepted March 27, 1981. This research was funded by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN, under Research Grants R800861 and R803950.